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Effects of Transportation Stress on Haematological Parameters of Blackchin Tilapia *Sarotherodon melanotheron*

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Abstract: Effects of transportation induced stress, on the haematology of *Sarotherodon melanotheron* was investigated. One hundred and twenty adult male and female *S. melanotheron* mean weight (44.24 g±0.26) and mean length (14.12 cm±0.34) were harvested at low tide from the recruitment ponds of the African Regional Aquaculture Centre, Brackish water research fish farm, Buguma, Rivers State, Nigeria and transported, live in 2 groups (with and without oxygen) over a distance of about 50 km by road. The results obtained showed a significant reduction ($p<0.05$) in mean values of Haemoglobin (Hb), Packed Cell Volume (PCV) Leukocrit (LCT), Red Blood Cell (RBC) and the Thrombocytes (Thromb). While the numbers of White Blood Cell (WBC), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Neutrophils (Neut) Lymphocytes (Lymph) and Monocytes (Mon) increased. The variation in these parameters, were more observable in the fish transported without oxygen, than the aerated samples. There were immediate and delayed mortalities which was very high in the fish transported without oxygen.

Key words: Transportation, haematology, oxygen, black chin tilapia, stress

INTRODUCTION

The transportation of the fish to the waters in which they are to be stocked is an extremely important aspect of fish culture and management (Beraka, 1986). Transportation usually involves moving large number of fish in small amount of water, depending on the species, over some distances (Orji,2005). It is a traumatic procedure that exposes fish to several adverse stimuli evoking hormonal, metabolic and haematological dysfunction in fish (Robertson *et al*., 1987). According to Pickering (1981) transport of fish is an inevitable procedure, which can elicit stress, leading to decreased fish performance. Also it is known to produce alterations of the peripheral leucocytes distribution such as *heterophilia* and lymphocytopenia (Ellsaesser and Clem, 1986; Maule and Shreck, 1990; Ainsworth *et al*., 1991) as well as increased susceptibility to diseases (Pickering and Pottinger, 1985; Maule *et al*., 1989) and in extreme cases leads mortality. (Akinrotimi, 2006). These changes are mainly stress induced by increased circulating corticosteroids, especially cortisol, which can alter different immune function, induce changes in plasma, tissue ion, metabolite levels and haematological features, (Iwama *et al*., 1998; Mommsa *et al*., 1999; Barton, 2002). It should be borne in mind that a very devastating effect of stress may occur during and after transportation of fish without apparent warning. This raises the question of proper monitoring of transportation induced stress in order to bring some relief to the fish by devicing a proper means of transporting fish.

Available information in literature, on the effects of transportation on the blood characteristics of fish include that of Coho Salmon *Oncorhynchus Kitsutch* (Specker and Shreck, 1980); Ranbow trout-*Salmo gairdneri* (Barton and Peter, 1982); Small mouth bass (Carmichael *et al*., 1983); Red drum -*Sceiaenops ocellatus* (Robertson *et al*., 1987). Chinook Salmon (Maule *et al*., 1989; Congleton *et al*., 2000); Matrinxa- *Brycon* cephalus (Carneiro and Urbinati, 2002); Nile tilapia *Oreochromis niloticus* (Orji, 2005). But none is available on Black chin tilapia *Sarotherodon melanotheron*, a common cultivable specie in the brackish water zone of Nigeria, that is usually transported, by fish farmers to the waters where they are to be cultured. Most often they arrive at the stocking site in poor conditions, if not properly manage can lead to poor performance after wards. Hence, the need to carry out this study, to assess the effects of transportation stress on the fish blood and compare the haematological response of this specie under two treatment (with and without oxygen).

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MATERIALS AND METHODS

One hundred and twenty adult male and female S. *melanotheron* mean weight (44.24 g±0.34 SD) and mean length (12.06 cm±0.41SD) were harvested from recruitment ponds of African Regional Aquaculture centre, brackish water Research fish farm. Buguma rivers State, Nigeria. They were immediately transported to Rivers State University of Science and Technology, Port Harcourt a distance of about 50 km away. The fish were transferred in two groups, (with and without oxygen), with three replicates of twenty fish each. The oxygenated sample were transported in enclosed oxygen bag, while the one without oxygen was in open circular plastic tank of 0.025 m^3.

Blood samples were collected from a total of 36 fish, that is 18 fish before and 18 fish after transportation, blood samples were obtained with heparinized plastic syringe, fitted with 21 gauge hypodermic needle and preserved in disodium salt of Ethylene Diamine Tetracetic Acid (EDTA) bottles for analysis. Physicochemical parameters of the waters in the recruitment ponds and in the experimental tanks were taken by using standard methods of APHA (1985).

Standard haematological procedures described by Brown (1980) were employed in the asserssment of the various blood parameters. Haemoglobin (Hb) was done by the cyanomethaemoglobin method, Packed Cell Volume (PCV) by Thrombocytes microhaematocrit method, Packed Cell Volume PCV) by microhaematocrit method by the Microwintrobe method, WBC was determined with the improved Neubauer counter, differential count was done on blood film stained with may Grumwald-Giemsa stain RBC was estimated using the relationship between Hb and PCV (Miale, 1982). The following indices: Mean Corpuscular Haemoglobin Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated according to Brown (1980). Leucocrit was done according to Wedemeyer *et al*. (1983).

Data obtained from the experimental fish were subject to analysis with the General Liner Model (GLM) of ANOVA at 0.05% probability and differences among means were separated with the significant difference using SAS soft ware.

RESULTS

Physcio-chemical parameter of the water before and after transportation were highly significant ($p<0.05$) except for temperature and salinity (Table 1). After transportation 70% mortality was recorded in fish

Table 1: Physico-chemical parameters of recruitment pond and experimental tanks (before and after transportation)

Parameters	Before transportation Mean±SD*	After transportation (With oxygen) Mean±SD*	(Without oxygen) Mean±SD*
Temperature°C	27.26±0.35^a	26.18±0.61^a	27.10±0.14^a
PH	6.82±0.24^a	6.05±1.21^b	5.59±0.69^b
N-NH_3(Mg L^{-1})	0.49±0.01^a	0.20±0.02^b	0.81±0.12^C
N-NO_2(Mg L^{-1})	0.0043±0.03^a	0.00040±0.01^a	0.0069±0.01^b
Dissolve oxygen (Mg L^{-1})	4.30±0.03^a	9.78±0.42^b	3.98±0.02^c
Sulphide (Mg L^{-1})	0.05±0.02^a	0.001±0.02^b	0.003±0.03^c
Salinity (0/00)	10.44±0.62^a	10.32±0.52^a	10.36±0.16^a

*Means values with different superscripts in the same row are significantly different ($p<0.05$)

Table 2: Haematological values of male black chin tilapia before and after transportation (Mean±SD)

				After transport					
	Before transportation			(With oxygen)			(Without oxygen)		
Parameters	Mean±SD*	Min	Max	Mean±SD*	Min	Max	Mean±SD*	Min	Max
Hb	6.52±0.35^a	4.25	7.34	6.32±0.21^b	3.89	6.54	4.01±0.21^c	2.11	5.12
PCV	22.12±0.24^a	17.10	23.10	20.12±0.41^b	16.14	21.21	16.21±0.41^c	14.21	18.16
Lct	7.01±0.46^a	2.60	9.21	6.21±0.12^b	2.40	6.51	5.21±0.62^c	2.01	5.81
WBC	28.12±0.72^a	19.00	40.00	29.11±0.36^b	19.12	36.71	31.12±0.41^c	20.16	39.12
RBC	2.44±0.12^a	1.60	2.86	2.39±0.42^b	2.58	2.81	1.43±0.21^b	1.04	1.96
MCHC	31.56±0.1.21^a	29.11	40.21	33.12±0.2^b	29.01	41.12	35.14±0.41^c	29.18	41.21
MCH	22.12±0.1.16^a	79.21	26.22	23.68±0.12^b	18.11	28.11	29.21±0.26^c	19.15	31.12
MCV	82.34±1.61^a	62.40	108.12	85.18±0.14^b	60.11	109.121	90.32±0.41^c	62.14	112.10
Thromb	1.79.41±4.32^a	69.12	240.22	170.21±0.12^b	70.12	242.62	159.12±0.32^c	100.12	171.12
Neut	33.61±1.21^a	18.78	43.10	40.38±0.46^b	19.68	44.76	46.22±1.21^c	20.11	49.12
Lynp	47.21±01.18^a	31.21	69.10	50.32±0.88^b	30.20	61.21	57.23±0.66^c	31.21	71.21
SMon	2.61±0.18^a	1.26	3.71	2.96±0.63^b	1.46	3.91	3.61±0.22^c	1.71	4.11

Key: Hb: Haemoglobin (gd L^{-1}), Ht: Haematocrit (%), Lct: Leucorit (%) MCV: Mean Corpuscular Volume (fL)wbc: White Blood Count ($\times 10^9$cells L^{-1}); RBC-Red Blood Cells ($\times 10^{12}$cells L^{-1}), MCH: Mean Corpuscular Haemoglobin (pg). MCHC: Mean Corpuscular Haemoglobin Concentration (g dL L^{-1}), Plt-Platelets ($\times 10^9$cells L^{-1}) Neut: Neutrophils (%), Mono: Monocytes(%), *Means values with different superscripts in the same row are significantly different ($p<0.05$)

Table 3: Haematological values of female black chin tilapia before and after transportation (Mean±SD)

	Before transportation			After transport (With oxygen)			After transport (Without oxygen)		
Parameters	Mean±SD*	Min	Max	Mean±SD*	Min	Max	Mean±SD*	Min	Max
Hb	7.70±0.62^a	5.60	8.30	7.21±0.34^a	4.10	8.10	5.02±1.21^b	3.90	6.21
PCV	23.14±1.21^a	19.01	27.20	22.16±0.19^a	18.26	26.14	18.11±0.91^b	16.21	22.12
Lct	3.60±1.42^a	5.60	11.90	8.10±0.16^a	5.80	11.10	6.14±1.72^b	2.61	7.12
WBC	31.02±2.12^a	16.00	38.10	33.04±0.34^b	17.10	39.14	39.12±1.73^c	17.00	44.32
RBC	2.67±1.12^a	2.38	2.96	2.02±0.14^b	2.24	2.72	1.14±0.62^c	1.01	2.02
MCHC	33.64±4.12^a	22.79	40.10	35.12±6.78^b	24.12	42.11	38.42±6.12^c	25.62	45.29
MCH	29.61±3.12^a	23.17	35.62	36.14±7.71^b	29.12	41.12	40.12±7.36^c	24.12	49.12
MCV	90.12±11.30^a	68.62	112.64	100.12±6.12^b	64.12	120.31	120.46±8.12^c	66.21	140.16
Thromb	165.21±18.24^a	141.21	192.00	150.16±10.12^b	142.39	181.12	110.21±6.02^b	88.61	130.99
Neut	36.92±6.21^a	23.42	44.76	41.21±1.21^b	26.12	48.29	50.11±1.12^b	29.12	61.12
Lymp	51.86±6.24^a	38.21	61.21	54.96±1.71^b	39.42	63.41	60.62±3.14^b	41.12	73.14
Mon	1.89±0.62^a	1.12	2.46	2.61±1.01^b	1.36	3.00	3.79±1.41^b	1.99	4.12

Key: Hb: Haemoglobin (gd L^{-1}), Ht: Haematocrit (%), Lct: Leucorit (%) MCV: Mean Corpuscular Volume (fL)wbc: White Blood Count ($\times 10^9$cells L^{-1}); RBC-Red Blood Cells ($\times 10^{12}$cells L^{-1}), MCH: Mean Corpuscular Haemoglobin (pg). MCHC: Mean Corpuscular Haemoglobin Concentration (g dL L^{-1}), Plt-Platelets ($\times 10^9$cells L^{-1}) Neut: Neutrophils (%), Mono: Monocytes(%), *Means values with different superscripts in the same row are significantly different ($p<0.05$)

Table 4: Mean value of haematological parameters of Black chin tilapia, transported with and without oxygen

	Before transportation			After transport (With oxygen)			After transport (Without oxygen)		
Parameters	Mean±SD*	Min	Max	Mean±SD*	Min	Max	Mean±SD*	Min	Max
Hb	7.11±1.12^a	4.25	8.30	6.72±0.42^b	3.89	8.10	4.52±0.96^c	2.11	6.21
PCV	22.63±1.22^a	17.10	27.20	21.11±0.12^b	16.14	26.14	17.16±0.28^c	14.21	22.21
Lct	7.81±1.32^a	2.60	11.90	7.22±0.26^a	2.40	11.10	5.68±0.14^b	2.01	8.12
WBC	29.57±1.36^a	16.00	40.00	31.75±0.26^b	17.10	39.14	35.17±0.86^c	17.00	44.32
RBC	2.55±1.12^a	1.60	2.96	2.23±0.62^a	1.58	2.81	1.31±0.71^c	1.01	2.02
MCHC	32.60±3.40^a	22.79	40.21	34.12±6.71^b	22.79	42.12	36.78±0.91^c	25.62	45.29
MCH	25.86±1.21^a	19.21	35.62	30.12±0.62^b	18.11	41.12	34.67±0.11^c	19.16	49.12
MCV	86.23±10.11^a	62.40	112.64	92.64±0.36^b	60.12	120.31	105.39±6.12^c	62.14	140.16
Thromb	172.31±16.22^a	69.12	240.22	160.19±0.71^b	70.12	242.63	134.67±18.12^c	88.61	171.11
Neut	35.27±18.61^a	18.78	44.76	40.79±0.86^b	19.68	48.29	48.17±3.64^c	20.11	61.12
Lymp	49.53±6.12^a	31.21	61.21	52.64±0.12^b	30.20	63.41	58.93±9.11^c	31.21	73.14
Mon	2.25±0.69^a	1.12	3.71	2.79±0.42^b	1.46	3.91	3.70±0.96^c	1.71	4.12

Key: Hb: Haemoglobin (gd L^{-1}), Ht: Haematocrit (%), Lct: Leucorit (%) MCV: Mean Corpuscular Volume (fL)wbc: White Blood Count ($\times 10^9$cells L^{-1}); RBC-Red Blood Cells ($\times 10^{12}$cells L^{-1}), MCH: Mean Corpuscular Haemoglobin (pg). MCHC: Mean Corpuscular Haemoglobin Concentration (g dL L^{-1}), Plt-Platelets ($\times 10^9$cells L^{-1}) Neut: Neutrophils (%), Mono: Monocytes(%), *Means values with different superscripts in the same row are significantly different ($p<0.05$)

transported without oxygen, while 10% was recorded in oxygenated sample. Before transportation the values of Hb, PCV, LCT, WBC, RBC, MCHC, MCH, MCV, Thrombocytes, neutrophils and lymphocytes in females were higher than the males. But monocytes values in males was higher than the females (Table 2 and 3).

Transporting fish over some distance, at a given period of time exerted some levels of change on the haematological parameters (Table 4) resulting in significant reduction ($p<0.05$) in mean values of Hb, PCV, RBC and thrombocyties; while the values of WBC, MCHC,MCV, neutrophils, lympocytes and monocytes increased significantly (Table 4).

After transportation the values of Hb, PCV, RBC and thrombocytes, reduced, while WBC, MCHC, MVC, neutrophils hymphocytes and monocytes increased, these variations were more pronounced in female than male (Table 2 and 3) and more observable in fish transported without oxygen then the created sample.

DISCUSSION

It is a well known fact, that stressors are real and perceived challenges to fish ability to meet its metabolic needs. Recently, haematological techniques are being used to determine the sub lethal effects of these stressors during the clinic diagnosis of fish physiology (Bhagwant and Bhikajee, 2000). According to Pickering (1981) the haemotological response of fish to stress is directed by the nature of the stress itself, that is, specific stress elicit specific responses. The psychological aspects of stress appear to be important in terms of physiological responses to stressful situations and perhaps the ability of the fish to resist exhaustion or achieve compensation. Specker and Shreck (1980) observed that salmonids subjected to transportation stress display an immediate GAS (General Adaptation Symdrome) stress response, which is very crucial in establishing the condition of the fish with respect to survival during transportation or ability to perform after liberation.

This study indicated that transportation of *S melanotheron* over a long distance induces a variety of distortions and anomalies in their blood characteristics. The decrease observed in the number of Hb and RBC, which was more pronounced and observable in the treatment without oxygen then the aerated samples, is in agreement with findings of Carmichael (1984) and Rottman *et al.* (1992) who reported the same in large mouth bass transported over a long distance. This may be attributed to a stress mediated condition, which trigger the reduction in production of new erythrocytes form the erythropoietic tissue. This reduction suggests an adaptation syndrome of *S. melanotheron* in reducing their oxygen demand, metabolic rate and energy production to meet up with the challenge imposed on it by transportation stress. Similar observation and explanation had been reported by Robertson *et al.* (1987). The overall results obtained, showed a decrease in the value of PCV this conforms to results obtained by Orji (2005) who noted a reduction in the value of PCV of *Oreochromis niloticus* transported without oxygen over a long distance.

The reduction in the number of Thrombocytes recorded in this research, is in agreement with the report of Urbinati who reported similar observation in Matrinxa (*Brycon cephalus*), this may be due to Iymphopenia, which triggers the release of lymphocytes in the lymphoid tissue, which lead to reduction in the number of thrombocytes. The observed increment in the MCV, MCH and MCHC, indicate that transportation stress may have interfered with the normal physiology of RBC as observed by Okechukwu *et al.* (2007) on *Clarias gariepinus* exposed to acute nominal doses of Chlorpyrifos-ethlyl. The increased in the number of monocytes and neutrophils recorded in this present study, agree with the findings of Gabriel *et al.* (2007) who observed same in *S. melanotheron* under confinement stress, this is probably due to functional activation of the haemopoietic tissue (Peters and Schwarzer, 1985) which release more of these monocytes and neutrophils to combact the external stressor. Rowley *et al.* (1988) reported that the granulocyte function of neutrophils and monocyte, is an important non- specific defense in fish, which serve as a first line of cell-mediated defense in the face of any external stressor.

CONCLUSION

The physiological status of fish after transport is only temporarily affected, especially, if it is properly transported by using oxygen bag. However, if consecutive disturbances occur before full recovery of the immunological organs, a stronger immuno depression probably resulting in increased mortality rates is produced. Transporting fish in oxygen bags, moderates the effects of stress, most especially in brackish water species.

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